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(54) Title: HISTAMINE DERIVATIVES AND METHODS FOR THEIR USE AS IMMUNOMODULATORS

(57) Abstract

The present invention provides histamine derivatives and methods for using histamine derivatives as immunomodulators and in immunotherapeutics. More specifically, the present invention provides methods for inhibiting at least a portion of an antigen specific antibody response and/or a portion of a T cell proliferative response by the immune system of a mammal comprising administering to said mammal an effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor.

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HISTAMINE DERIVATIVES AND METHODS FOR THEIR USE AS IMMUNOMODULATORS

FIELD OF INVENTION

The invention relates generally to histamine derivatives and methods for modulating the immune system of mammals and more particularly, to methods for modulating the immune system using compositions comprising histamine derivatives.

BACKGROUND OF THE INVENTION

With the increased level of understanding concerning the immune response process in mammals, there, is a growing awareness that certain molecules are generally nonspecific as to their effects on single cell types in a mixture of cells. A critical need exists for agonists that are effect or cell specific.

Histamine is a small molecule that has been shown to have a significant role in the immune response process in mammals. However, its ubiquitous effects on many cells that have receptors for histamine limits its possible immunotherapeutic use. Histamine derivatives that are tissue directed or effect specific would significantly aid in determining the role of histamine in immune modulation and produce valuable immunotherapeutics.

Histamine can substantially modulate models of immune responses in mammals, particularly models of delayed hypersensitivity and T and B cell functions. Histamine is synthesized during different phases of response to antigen and is able directly or indirectly to effect further responses to antigen. It is possible that the concentration of histamine in tissue during inflammation and immune response can modify the function of a number of lymphoid cells. Although these effects may be substantial, the direct effect on single cell types in a mixture of cells cannot be determined unless the agonists are effect or cell specific. Ubiquitous effects of agonists on all cells that have receptors for histamine would limit any immunotherapeutic use of histamine. See Khan, et al., Clin. Immunol. Rev., (1985) Melmon, et al., Am. J. Med., (1981) 71:100, and Roclin et al., Cell Immunol., (1978) 37:162.

Histamine is an autacoid as are catecholamines, prostaglandins and some peptides, e.g., bradykinin and probably lymphokines. Autacoids differ from hormones in that they are made at their local sites of action and they can be made in a variety of tissues. Autacoids play an important role in mediating inflammation. During inflammation, certain events may occur which include: protein denaturation, lowering of local pH, release of "new peptides" and lysosomal enzymes, and the like. Such events create a setting in which the immune system should not overreact to the new products. Yet, despite the ability of inflammation to generate likely immunogens, the inflammatory process usually is not accompanied or followed by grossly abnormal immune responses. Autacoids appear to somehow modulate this response.

Autacoids affect natural suppressor cells, T cell subsets and B cells during various stages of immunity. Receptors for autacoids are non-randomly distributed (in number and affinity for agonist) on cells that carry out immune functions. Precursor B cells do not appear to have histamine and catecholamine receptors, while B cells committed to produce antibodies do. T suppressor (T_S) cells modulate the CAMP responses of T helper (T_h) and T cytolitic (T_C) cells to histamine. Mitogens alter responsiveness of these cells to histamine. In some cells biologic response is inhibitory (e.g. reduced release of antibody from B cells: inhibition of lymphokine release or lysis of target cells by T effector cells and inhibition of release of histamine from MAST cells); in other the response enhances immune function (e.g. enhanced suppression by natural suppressor and T_S cells or T helper (T_h) cell proliferation). The autacoids seem to be enhancing selected early events in immune response (e.g. enhanced suppressor function) while inhibiting later phases of phenotypic manifestations (e.g. release of lymphokines or antibodies) of immunity.

The appearance of naturally occurring suppressor cells in the spleens of neonatal or irradiated mice may have a key role in induction of immune tolerance. See Strober et al., Ann. Rev. Immunol. (1984) 2:219; Hertel-Welff et al., J. Immunol. (1984) 133:2791; Okada et al., J. Expt. Med. (1982) 156:522; and Okada et al., J. Immunol. (1982) 129:1892. These cells are related to NK cells in terms of their surface phenotype but differ in function. The natural suppressor cells appear briefly during the early maturation of lymphoid tissue but can be induced in adults by total lymphoid irradiation. The cells have the unique feature of inhibiting the antigen-specific cytolytic arm of alloreactive immune

response but leave the antigen-specific cytolytic arm intact. In this way, alloreactions in the regulatory milieu of natural suppressor (NS) cells generate large numbers of antigen-specific suppressor cells that in turn maintain tolerance *in vivo*. Thus, the natural suppressor cells may play an important role in preventing the development of host versus graft and graft versus host diseases in allogenic bone marrow chimeras, and in immune tolerance in the neonatal and total lymphoid irradiated (TLI) mice.

Histamine activates human T_S cells and enhances the suppressive ability of murine NS cells *in vitro*. See Khan et al., J. Immunol. (1985) 134:4100 and Sansoni et al., J. Clin. Invest. (1985) 75:650. After pretreatment of both human T_S cells (leu2., 9.3) with histamine, both phytohemagglutinin-induced T_h proliferation and pokeweed mitogen-induced B cell differentiation were inhibited. The effects were mediated via H_2 receptors. Natural suppressor cells can be propagated and cloned in long-term tissue culture and cause nonspecific suppression in both *in vitro* and *in vivo* models of mixed leukocyte reactions.

Therefore, methods using histamine derivatives that have little or no systemic effects in immune modulation and immunotherapeutics would be advantageous.

SUMMARY OF THE INVENTION

The present invention provides histamine derivatives preferably having the following formula:

Formula 1: $His\text{-}NH\text{-}(CH_2)_nCONH\text{-}phi\text{-}CF_3$

where n=2-10, more preferably wherein n=3-6, and most preferably wherein n=5;

and Formula 2: $His\text{-}NH\text{-}CHCH_3\text{-}(CH_2)_nCONH\text{-}phi\text{-}CF_3$

where n=2-10, preferably 3-6, more preferably 4.

The invention further provides methods for inhibiting at least a portion of an antigen specific antibody response and/or a portion of a T cell proliferative response by the immune system of a mammal comprising administering to the mammal an effective amount of a composition administering to the mammal an effective amount of a composition comprising at least one histamine derivative

having binding specificity for at least one histamine receptor e.g. H_1, H_2, H_3, or H_X and a pharmaceutically acceptable carrier or diluent. The invention also provides methods of treating T cell mediated diseases and graft rejection in an individual by administering to the individual a therapeutically effective amount of a composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor and a pharmaceutically acceptable carrier or diluent.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a is a graphic representation depicting the results of an ELISA assay showing the total anti h-Mb IgG antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day before (day-1) antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment.

Fig. 1b is a graphic representation depicting the results of an ELISA assay showing the anti h-Mb IgG_1 antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day before (day-1) antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

Fig. 1c is a graphic representation depicting the results of an ELISA assay showing the anti h-Mb IgG_2 antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day (day -1) before antigen treatment with 100 ug h-Mb in Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

Fig. 1d is a graphic representation depicting he results of an ELISA assay showing the anti h-Mb IgG_{2b} antibody response in two groups of 2-6 DBA/2 mice which had been treated with either saline PBS or 36 mg/kg of Compound 3 intravenously one day (day -1) before antigen treatment with 100 ug h-Mb in

Complete Freund's Adjuvant (CFA) injected intravenously and two days following h-Mb antigen treatment (day+2).

Fig. 2 is a graphic representation depicting a T cell proliferative response assay showing the effect of Compound 3 on spW-Mb specific T cell proliferation in mice. Two groups of 4 DBA/2 mice were given either 32 mg/kg Compound 3 in buffer or PBS control (on day (-2) and day (-1) intravenously), the mice were primed with 100 ug spW-Mb intravenously on day 0, lymph nodes were pooled and harvested on day 8.

Fig. 3 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freunds Adjuvant (CFA) on day 0 and 100 ug of h-Mb in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and sera was assayed for h-Mb specific IgG. The mean antibody binding from 5 mice is shown.

Fig. 4 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freunds Adjuvant (CFA) on day 0 and 100 ug of h-Mb in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and sera was assayed for h-Mb specific IgG2a. The mean antibody binding from 5 mice is shown.

Fig. 5 is a graphic representation of an ELISA assay showing the IgG anti h-Mb antibody response in mice which had been treated subcutaneously (sc) with 35 mg/Kg Compound 1 or Compound 3 or saline (PBS) control on day-1 and day 2, and 100 ug of h-Mb in complete Freunds Adjuvant (CFA) on day 0 and 100 ug of h-Mb in Incomplete Freund's Adjuvant (IFA) on day 21. Mice were bled on day 33 and sera was assayed for h-Mb specific IgG2b. The mean antibody binding from 5 mice is shown.

Fig. 6 is a graphic representation of an ELISA assay showing the effect of Compound 1 and Compound 3 on h-Mb specific T cell proliferation. 3 mice were given either 35 mg/kg or PBS (control on day -2 and day -1 intravenously. the mice were primed with 100ug h-Mb/CFA subcutaneously on day 0. Lymph nodes were pooled and harvested on day 7. proliferation of lymph node T cells is shown.

Fig. 7 is a graphic representation showing the effect of Compound 1 only, Compound 3 only, or Compound 1 and Compound 3 together on the incidence of diabetes in NOD mice. 10 mice were treated subcutaneously with 35 mg/Kg Compound 1 only, Compound 3 only, Compound 1 and Compound 3 together, or saline (PBS) on days 90 and 91 of life. The incidence of diabetes was measured by serum glucose levels.

Fig. 8 is a graphic representation showing the effect of Compound 1 only, Compound 3 only, or Compound 1 and Compound 3 together on the incidence of diabetes in NOD mice, 10 mice were treated subcutaneously with 35 mg/Kg Compound 1 only, Compound 3 only, Compound 1 and Compound 3 together, or saline (PBS) on day 76 of life at each data point shown. The incidence of diabetes was measured by serum glucose levels.

Fig. 9 is a graphic representation of the effect of Compound 1 or Compound 3 on the IgM response of mice which were treated subcutaneously with 35 mg/Kg Compound 1, Compound 3 or PBS (Control) on day 0 and day2 and 100 ug of h-Mb/CFA on day 0, the mice were bled on day 7 and sera was assayed for h-Mb specific IgM. The mean antibody binding from 5 mice is shown.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides histamine derivatives (also known as a histamine congeners) having the formulas:

His-NH-$(CH_2)_n$CONH-phi-CF_3 (Formula 1)

where n=2-10, preferably 3-6, most preferably 5 and

His-NH-$CHCH_3$-$(CH_2)_n$CONH-phi-CF_3 (Formula 2)

where n=2-10, preferably 3-6, more preferably 4.

Histamine derivatives of particular interest have the following formulas:

His-NH-$(CH_2)_5$CONH-phi-CF_3 (Compound 3)
His-NH-$CHCH_3$-$(CH_2)_3$CONH-phi-CF_3 (Compound 2)
His-NH-$CHCH_3$-$(CH_2)_4$CONH-phi-CF_3 (Compound 1)

The histamine derivatives of the inventions may be synthesized by various methods according to procedures well known in the art. A discussion of the synthesis of the above-described histamine derivatives can be found in U.S. Patent No. 4,996,221, incorporated herein by reference.

The histamine derivatives may be purified by conventional purification techniques, such as crystallization, or by chromatographic techniques, such as column chromatography, high performance liquid chromatography, preparative thin-layer chromatography, or the like.

It is understood that the subject invention includes derivatives of histamine wherein histamine is connected to a linking group to an amino acid of poly(amino acid) molecule thereby defining a conjugate. The histamine derivative may be linked to a carrier such as polypeptides, proteins, glycoproteins or derivatives thereof (all included within the name poly(amino acid).

The conjugates may serve a variety of functions, changing the physiological character of the histamine derivative, acting as immunogens, providing for cell specific binding and the like. Depending on the purpose of the conjugate, the nature of the histamine derivative may be modified to lesser or greater degrees by adding additional functionalities, substituting groups or the like. Particularly for the production of antibodies from immunogens, a group may be substituted for another group, e.g. methyl or trifluoromethyl with carboxyl. Also, in the case of immunogens, substitution at histamine or intermediate the ends of histamine derivative may be desirable.

The conjugates may be bonded through a wide variety of functionalities to form amide, methyleneamine, thioether, disulfide, sulfonamide, azo, amidine, etc. The particular functionality chosen will depend upon the purpose of the conjugate, ease of synthesis, stability of the linking functionality, affect of the linking group on the physical chemical like.

The number of histamine derivatives per carrier may be one, or any number greater than one. The number of histamine derivatives per carrier molecule is dependent upon the number of appropriate functional groups in the carrier and the stoichiometry used during the coupling reaction.

Syntheses routes are well known in the art. One method would involve the preparation of appropriate histamine derivatives where the extended amine side chain or other location on histamine has a suitable functional group. One or more functionalized histamine derivatives are then, in turn, coupled to appropriate side chains of the carrier. Alternatively, a method of synthesis may involve the initial modification of the carrier by coupling the derivative group moiety containing a further functional group reactive with histamine directly to the carrier side chain. The resulting carrier derivative is then coupled directly to the histamine, for example, by a reductive amination reaction to produce the conjugate.

A number of specific physiologic and immunologic factors lead to non-random expression of histamine receptors and their subtypes on various cells responsible for the immune response. Table 1 summarizes the histamine receptor activity of various derivatives of histamine:

Dominant Interactions of Gengeners with Histamine Receptors on Human Lymphocytes, Monocytes and Neutrophils

Table 1

Compound #	H_1	H_2	H_3	H_X
1	++	++	Anti H_3	+++
2	+++	-	NT	+
3	-	+++	NT	++
Histamine	+	+	+	Anti H_X

NT = not tested

Compound 3 has been shown to have H_2 receptor activity but not H_1 receptor activity, and further has some H_X activity. As referred to herein, a

compound which has receptor activity can bind to a specific receptor. It is believed that compounds having H_1 receptor activity enhance the suppressive capacity of the natural suppressor cells via H_1 receptor mediated mechanisms. It is believed that compounds having H_2 receptor activity effect immune call modulation by stimulating intracellular accumulations of CAMP.

Hx receptors are believed to be found only in human monocytes, neutrophils, and HL-60 cells that have been transformed. The H_x receptor system is believed to play a role in mediating certain immune responses such as suppressing IL-4 secretion from T helper cells, inhibiting natural T-killer cell activity, enhancing suppression caused by natural suppressor cells and increasing the calcium flux in HL-60 cells and human peripheral blood lymphocytes (PBL). Therefore, compounds having H_x receptor activity are believed to have an effect on these processes.

Although different autacoids certainly share a number of molecular mechanisms of effect and do produce similar effects of immunosuppression, the fine specificity of histamine receptors and their interactions with the congeners provide the potential for orchestrating the use of the likely complementary effects produced by combinations of congeners. In order to achieve a desired effect, it may be beneficial to provide a composition comprising two or more histamine derivatives. Preferably such a composition comprising two or more compounds is selected from the groups consisting of:

His-NH-$(CH_2)_5$-CONH-phi-CF_3 (Compound 3)
His-NH-$CHCH_3$-$(CH_2)_3$-CONH-phi-CF_3 (Compound 2)
His-NH-$CHCH_3$-$(CH_2)_4$-CONH-phi-CF_3 (Compound 1)

In one embodiment of the invention, the histamine derivatives of Formulas 1 and/or 2 are useful in methods of inhibiting at least a portion of an antigen specific antibody response by the immune system of a mammal. In particular, administration of an effective amount of at least one histamine derivative having binding specificity for at least one histamine receptor inhibits the production of IgG antibodies while it appears that the production of IgM antibodies are not inhibited. Administration of at least one histamine derivative of Formulas 1 and/or 2 results in inhibition of an antigen specific antibody response by the immune system of a mammal of at least about 60% inhibition of

the production of total IgG antibodies, of which at least 40% of the production of IgG1 antibodies are inhibited, at least 40% of the production of IgG2a antibodies are inhibited and at least 40% of the production of IgG2b antibodies are inhibited. In particular, the specific histamine derivatives, Compound 3 and/or Compound 1, will be of interest in inhibiting these antigen specific antibody responses.

Furthermore, at least one histamine derivative of Formula 1 is useful in inhibiting at least a portion of an antigen specific T cell proliferative response by the immune systems of a mammal. In particular, administration of an effective amount of such a histamine derivative inhibits T cell proliferation by at least 60% and preferably by at least 80%. The inhibition is dependent on the dose of the histamine derivative, can endure for extended periods of time, and can be prolonged by repeated dosing of the histamine derivative.

Another embodiment of the present invention provides methods of treating T cell mediated diseases or a method of treating graft rejection in an individual comprising administering to the individual a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative of Formulas 1 and/or 2 along with a pharmaceutically effective carrier or diluent. T cell mediated diseases which can be treated include but are not limited to diabetes, T cell leukemia, endotoxin induced food poisoning and mycosis fungoides. In particular, the specific histamine derivatives, Compound 3 and/or Compound 1, will be of interest in treating T cell mediated diseases or treating graft rejection in an individual.

The manner in which a composition comprising at least one histamine derivative of Formulas 1 and/or 2 is administered to a mammal varies widely in accordance with methods well known in the art. The composition is preferably administered with a physiologically suitable or pharmaceutically acceptable buffer as is known in the art and includes but is not limited to phosphate buffered saline (PBS). Suitable methods of administration include but are not limited to: orally, parenterally, by injections or the like. Pharmaceutically effective concentrations and dosages of compositions comprising at least one histamine derivative will vary widely, depending upon the purpose, host and particular derivative employed. Concentrations may vary from less than 10-1M, and preferably less than or equal to 10-3M. Suitable single pharmaceutically effective dosages of such compositions range from about 0.5 mg/kg body weight to about 100 mg/kg. A preferred range is from about 1 mg/kg to 50 mg/kg. Suitable

pharmaceutically effective daily total dosages range from about 1 mg/kg to 100 mg/kg.

The invention is further illustrated by the following non-limiting examples.

Example 1

The following experiment shows the inhibition of the production of IgG antibodies in mice as well as the inhibition of the production of the IgG antibody subclasses IgG1, IgG2a, IgG2b (which comprise at least a portion of the total IgG response), as a result of administering to the mice compound 3 and challenging the mice with a T cell mediated antigen, horse myoglobin (h-Mb), which elicits an IgG antibody response.

Anti-Horse Myoglobin ELISA

ELISA plates were coated with 10 µg/ml commercially available horse myoglobin (h-Mb) in PBS 50 µl/well overnight at 4°C. The plates were washed with PBS once. The plates were then coated with 50 µl BBA (1 mg/ml) and washed with PBS once. The plates were then coated with test serum diluted in PBS-Tween, 50 µl/well and incubated at 4°C overnight. The plates were then washed with PBS-Tween three times. Anti-IgG (1:2000) was added and diluted with PBS-Tween. For the IgG antibody subclasses, anti-IgG1, anti-IgG2a, anti-IgG2b was diluted and added to selected wells. Incubation was at 4°C for two hours. Washing was with PBS-Tween two times with 5 minutes between each wash. A 1 mg/ml OPD in citrate buffer solution pH5 was prepared and 10 µl of H202/10 ml was added to the buffer solution. 50 µl of buffer was added to each well and the plates were placed in the dark for about 10 minutes. The reaction was stopped with 50 µl of 5N H2SO4 in each well. The plates were then read on an ELISA reader.

Immunization

The following immunization protocol was repeated several times. The results have been collected in Figs. 1a-d.

This experiment was done using two groups of 2-6 DBA/2 mice (average weight 22 grams). One group of mice were given one treatment of Compound 3 intravenously one day before antigen treatment (day-1) and two days following

antigen treatment (day+2). The dose of Compound 3 was approximately 36 mg/kg in 100 µl of PBS. The other group of mice was given a saline control in 100 µl PBS. On day 0 the two groups of mice were injected with 100 µg h-Mb in 100 µl CFA intravenously (i.v.). The mice were then boosted fourteen days later with 100 µg of h-Mb in 100 µl of h-Mb in 100 µl IFA. The mice were bled 30 days later.

TABLE 2

Experimental Protocol

Group 1	5 mice	Day 0, Day 14 Day-1 & Day+2	100 µg h-Mb 36 mg/kg Compound 3
Group 2	5 mice	Day 0, Day 14 Day-1 & Day+2	100 µg h-Mb PBS Control

All sera was tested on the above described ELISA for IgG and IgG subclass antibody response to h-Mb. As shown in Figs. 1a-d, mice immunized with Compound 3 had a much lower total IgG response to h-Mb compared to the saline control mice here there was a significant IgG response. Furthermore, the mice immunized with Compound 3 showed a lower IgG1, IgG2a and IgG2b response than did the saline control mice in all of those same IgG subclasses.

Example 2

The following experiment shows T cell proliferation in mice in response to treatment with Compound 3 and challenge with commercially available sperm whale myoglobin (SpWMb) antigen. In this model, the response to SPWMb is mediated through CD4+ T cells.

T Cell Proliferation Study

Two groups of DBA/2 mice were tested. One group of four mice was a control. The other group of four mice were treated with Compound 3. Both groups of mice were immunized using approximately 100 µg per mouse of SpW-Mb plus CFA intravenously on day 0. On day-1 and day+2, one group of mice was given approximately 32 mg/kg Compound 3 in 100 µl PBS i.v.

On day 8, the mice were killed and the draining lymph nodes were removed and a single cell suspension was made. The cells were then plated out in a 96-well U-bottom plate at a concentration of 4x106 cell/ml in media containing 1% mouse serum. The cells were then challenged in vitro with antigen diluted at 1, 5, and 25 µM in media without serum. The plates were then placed in the incubator at 37°C. On day 4 the wells were pulsed with tritiated thymidine and on day 5 the plates were harvested. The results are shown in Fig. 2.

Example 3

Monoclonal Affinity Purification of *Fel d* I

Native *Fel d* I protein was purified from an extract of house dust as described by Chapman et al. Briefly, house dust (from vacuum containers used in homes with multiple cats) was extracted with PBS, then lyophilized and redissolved in water. The extract was applied to a column coupled with anti-*Fel d* I monoclonal antibody (hybridomas 6F9 and 1G4 were both provided by M. Chapman). The *Fel d* I was eluted from the column with 100 mM glycine pH 2.5 and was neutralized.

Direct Binding ELISA

For the IgG assay *Fel d* I was coated onto Immulon 2 (Dynatech, Chantilly, VA) 96-well plates by incubation of 50 µl/well of 2 µg/ml *Fel d* I in PBS overnight at 4°C. The wells were incubated with 0.5% gelatin in PBS at 37°C for one hour. Plates were washed three times with PBS-T (1 X PBS + 0.05% Tween 20). Sera were diluted in PBS-T. After incubation at room temperature for one hour and washing with PBS-T, the bound mouse antibody was detected by incubation with biotinylated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). Streptavidin conjugated to

horseradish peroxidase (Southern Biotechnology Associates) was added to detect antigen bound biotinylated antibody-complexes. TMB peroxidase substrate (Dirkegaard and Perry, Gaithersburg, MD) was used according to the directions supplied and resulting O.D. (450 nm) values were determined using an ELISA reader (Bio-Tek model #310, Winooski, VT). The serum titer is determined by 25% of the positive control.

H-Mb ELISA is carried out similarly, using isotype specific polyclonal reagents. Antigen bound IgE was detected similarly, but using biotinylated EM95-1, a rat monoclonal antibody specific for mouse IgE. Biotinylated goat anti-rat IgG (Dirkegaard and Perry) was used as an added signal amplification step in the IgE ELISA. Antigen bound IgM was detected similarly, but using anti-mouse IgM.

Culture Conditions for Proliferation Assays

The inguinal, paraaortic, and popliteal lymph nodes were removed from the animals seven days after antigen challenge. The cells from these organs were suspended by being forced through a stainless steel mesh with a glass pestle. The cells were washed two times in RPMI 1640 with 1% FCS before being cultured. All cells were cultured at 37°C in 5% CO_2 in RPMI-1640 with 10% FCS (#F4884, Sigma, St. Louis, MO), 100 U/ml penicillin G, 10 µg/ml streptomycin, 10 mM glutamine, and 5 x 10^{-5} M 2-ME. Cells were cultured in triplicate 0.2 ml wells for in 96 well plates at 4 x 10^6 cells/ml. Proliferation was measured by tritiated thymidine incorporation on Day 7.

The Effect of Histamine Congeners on Antibody Isotype

Recent work has demonstrated the ability of helper T cell subsets to augment different antibody isotopes. Murine TH_1 cells appear to stimulate the production of IgG_2 while TH_2 cells stimulate the production of IgG1 and IgE. Experiments are addressing whether histamine congeners can specifically effect different populations of helper T cell functions. Two different histamine congeners (Compound 1 and Compound 3 discussed earlier) have been compared for their ability to effect the antibody response to Horse myoglobin (H-Mb).

The IgM response specific for H-Mb was assayed on day 7 following an antigen priming with a day 0 and day 2 drug treatment (Fig. 9). There was no

detectable effect of 35 mg/Kg histamine congener on the antigen specific IgM made in response to the H-Mb priming.

A separate group of mice were treated with 35 mg/Kg histamine congener on day (-1) and day 2. These mice received H-Mb on day 0 and day 21. Sera (day 33) from these mice were assayed for H-Mb specific IgG. Fig. 3 demonstrates the ability of Compound 1 and Compound 3 to inhibit the production of H-Mb specific IgG. The same bleeds were assayed for H-Mb specific IgG2a (Fig. 4) and IgG2b (Fig. 5). Compound 3 appears to decrease the H-Mb specific IgG2a and IgG2b. This implies that the target of Compound 3 activity may be part of the TH_1 pathway. In contrast, Compound 1 does not effect the H-Mb specific IgG2a or IgG2b. The decrease in IgG found after Compound 1 treatment (Fig. 3) may reflect an effect on IgG1. The ability of Compound 1 to decrease IgG and IgE responses to *Fel d* I (data not shown) suggests that its target may be the TH_2 pathway.

The Effect of Histamine Congeners on T Cell Proliferation

Receptors for the Histamine congeners are present on most cells involved in the immune response. Experiments have been conducted to define the target of action of Compound 1 and Compound 3. Mice were primed with Mb and treated with drug on day (-2) and (-1) (iv). Draining lymph nodes were harvested and antigen specific proliferation was measured (Fig. 6). Antigen specific T cell proliferation is decreased by treatment of the mice with Compound 3. The data implies that the stimulation of specific T cell activation is affected by Compound 3. This appears to be consistent with the antibody isotype effect of Compound 3. The TH_1 activity is responsible for the majority of T cell proliferation *in vitro*.

Example 4

The Effects of Histamine Autacoids on an Autoimmune Disease Model

Insulin-dependent diabetes mellitus (IDDM or Type I Diabetes Mellitus) is an autoimmune disease and involves lymphocyte dependent inflammatory destruction of the insulin-producing beta cells in pancreatic islets of Langerhans. T lymphocytes have been implicated in the destruction of the pancreatic cells and autoantibody production is associated with the development of insulitis, the inflammatory lesion of IDDM. A strain of mouse (non-obese diabetic mice

referred to as NOD mice) develops a type of pancreatic lesion that closely resembles Type I diabetes in man. Many experiments using immunosuppressive drugs that showed efficacy in NOD mice predicted the value of immunosuppressives in people susceptible to Type I diabetes. Unfortunately, the imunosuppressives available for the treatment of Type I disease in susceptible patients are efficacious but only during the time they are given. Those agents, with the possible exception of anti-CD4 monoclonal antibody, are too toxic to give continuously for prolonged periods.

The experiments described herein focus on the effects of compounds 1 and 3 on the progress of hyperglycemia and the development of insulitis. The experiments focus on the following issues:

1. Whether short courses (two doses) of either drug or the drugs combined alter the onset of hyperglycemia and occurrence of death;

2. Whether protracted dosing of drug at weekly intervals alter the onset of IDDM in any more definitive way than short courses; and

3. Whether intermittent dosing of the two drugs retard or modify the onset or severity of the inflammatory lesion of the pancreas. It is believed that the immunosuppressive effects of the autacoid (histamine congeners) could influence the course of the disease because Compound 3 is capable of inhibiting T Cell proliferation to antigen. It should thus be able to limit a T cell-mediated cytolytic event.

The effects of short term treatment with compounds 1, 3 or 1 plus 3 were tested in groups of 10 female NOD mice. The drug (35 mg/Kg was given subcutaneously) for 2 consecutive days for a cumulative dose of 70 mg/Kg of compounds 1 or 3 and 140 mg/Kg of combined compounds 1 and 3. The drug was given on days 90 and 91 (Fig. 7). Suppression of the appearance of the hyperglycemia was most pronounced in the groups treated with Compound 3 or 1 plus 3. It is quite likely that the apparent effects of Compound 1 plus 3 is caused by the additive H_2 effects of the two drugs.

The effects of long term treatment with histamine congeners on the development of hyperglycemia in NOD mice was tested in groups of 10 mice per group. The drugs were administered subcutaneously as Compound 1, or 3 or 1

plus 3 in the same doses used above. Drug or control treatment was started on day 76 and repeated at the same dose/Kg 14 days later and then at weekly intervals. Fig. 8 shows the absence of any disease in the group treated with compounds 1 plus 3 up to day 140.

In summary, the Examples show that histamine congeners are potent immunosuppressants and each has a different potential mechanism of immunomodulation. Compound 1 suppresses T cell dependent IgE, IgG1 (but not IgM, IgG2a or IgG2b) antibody responses. In various antigenic responses, Compound 3 suppresses IgG1, IgG2a and IgG2b but not IgM responses. The effects of Compound 3 on IgE will be tested. Only Compound 3 appears to directly suppress T cell proliferation to specific antigen at the doses tested. The suppression of antibody production is transferable and also can be seen after the response to the antigen is established.

Histamine congeners have similar immunosuppressive effects in the mouse model of human IDDM. Congener treatment delays the onset of hyperglycemia and insulitis in NOD mice. Since treatment with compounds 1 and 3 but not Compound 1 alone results in the greatest effect so far determined, these results indicate that: H_1 and H_2 receptor effects may be needed and cooperative in the effects on the NOD mouse or the apparent cooperation may simply be additive H_2 effects contributed separately by the two drugs; dose dependence and blocking effects of H_1 or H_2 blockers will be studied to determine the pharmacologic mechanism by which the experiments work; the cellular mechanism by which the autacoids work are not established by the selectivity of the effects on isotopes and actions of T cell proliferation suggest the target for Compound 3 could be a TH_1 cell.

Although this invention has been described with reference to its preferred embodiments, other embodiments can achieve the same results. Variations and modifications to the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents that follow in the true spirit and scope of the invention.

What is claimed:

1. A compound having H_2 receptor activity but not H_1 receptor activity.

2. The compound of claim 1 having H_x receptor activity.

3. The compound of claim 1 comprising a histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10

4. The compound of claim 3 wherein n=5.

5. The compound of claim 2 comprising a histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10

6. The compound of claim 5 wherein n=5.

7. The compound comprising a histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10.

8. The compound of claim 7 wherein n=5.

9. A therapeutic composition comprising a histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10 wherein n=2-10. and a pharmaceutically acceptable carrier or diluent.

10. The compound of claim 9 wherein n=5.

11. A composition comprising at least two compounds wherein each compound is different and is selected from the group consisting of
His-NH-$(CH_2)_5$CONH-phi-CF_3
His-NH-$CHCH_3$-$(CH_2)_3$CONH-phi-CF_3
His-NH-$CHCH_3$-$(CH_2)_4$CONH-phi-CF_3 .

12. A method for inhibiting at least a portion of an antigen specific antibody response by the immune system of a mammal comprising administering to said mammal an effective amount of a composition comprising at least one histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10.

13. The method of claim 12 wherein n=5.

14. The method of claim 12 wherein production of IgG antibodies by the immune system of the mammal is inhibited by at least about 60%.

15. The method of claim 12 wherein production of IgG1 antibodies by the immune system of the mammal is inhibited by at least 40%.

16. The method of claim 12 wherein production of IgG2a antibodies by the immune system of the mammal is inhibited by at least about 40%.

17. The method of claim 12 wherein production of IgG2b antibodies by the immune system of the mammal is inhibited by at least about 40%.

18. The method of claim 12 wherein the composition comprises at least one histamine derivative administered to the mammal in a dosage of less than or equal 50 mg/kg.

19. A method for inhibiting at least a portion of an antigen specific T cell proliferative response by the immune system of a mammal comprising administering to said mammal an effective amount of a composiion comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n-2-10.

20. The method of claim 19 wherein n=5.

21. The method of claim 19 wherein antigen specific T cell proliferation is inhibited by at least 60%.

22. The method of claim 19 wherein antigen specific T cell proliferation is inhibited by at least 80%.

23. A method of treating T cell mediated disease in an individual, comprising administering to the individual a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10.

24. The method of claim 23 wherein n=5.

25. The method of claim 23 wherein the T cell mediated disease is selected from the group consisting of: diabetes, T cell leukemia, endotoxin induced poisoning and mycosis fungoides.

26. A method of treating graft rejection in an individual, comprising administering to the individual a therapeutically effective amount of a therapeutic composition comprising at least one histamine derivative having binding specificity for at least one histamine receptor, said histamine derivative having the formula:
His-NH-$(CH_2)_n$CONH-phi-CF_3 wherein n=2-10.

27. The method of claim 23 wherein n=5.

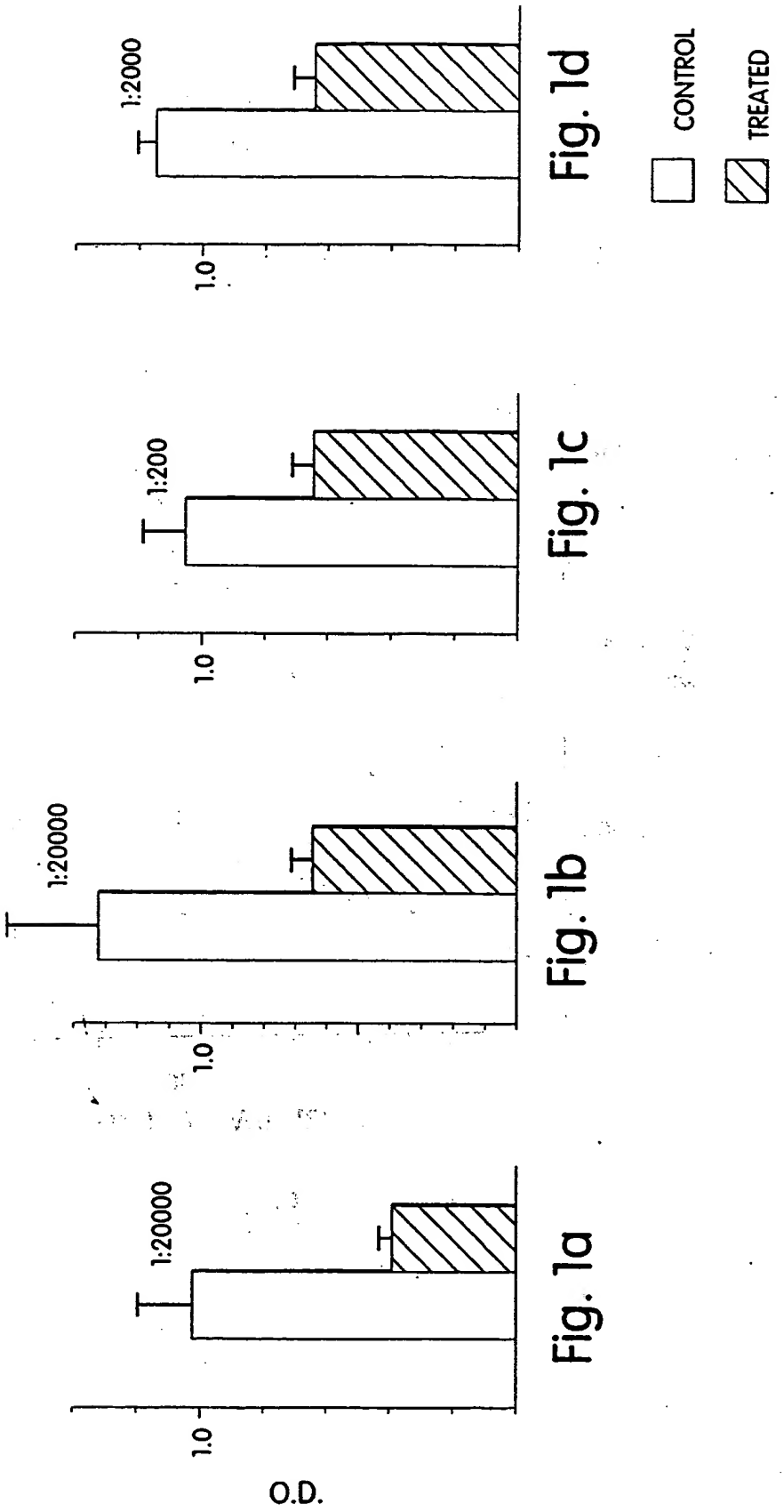
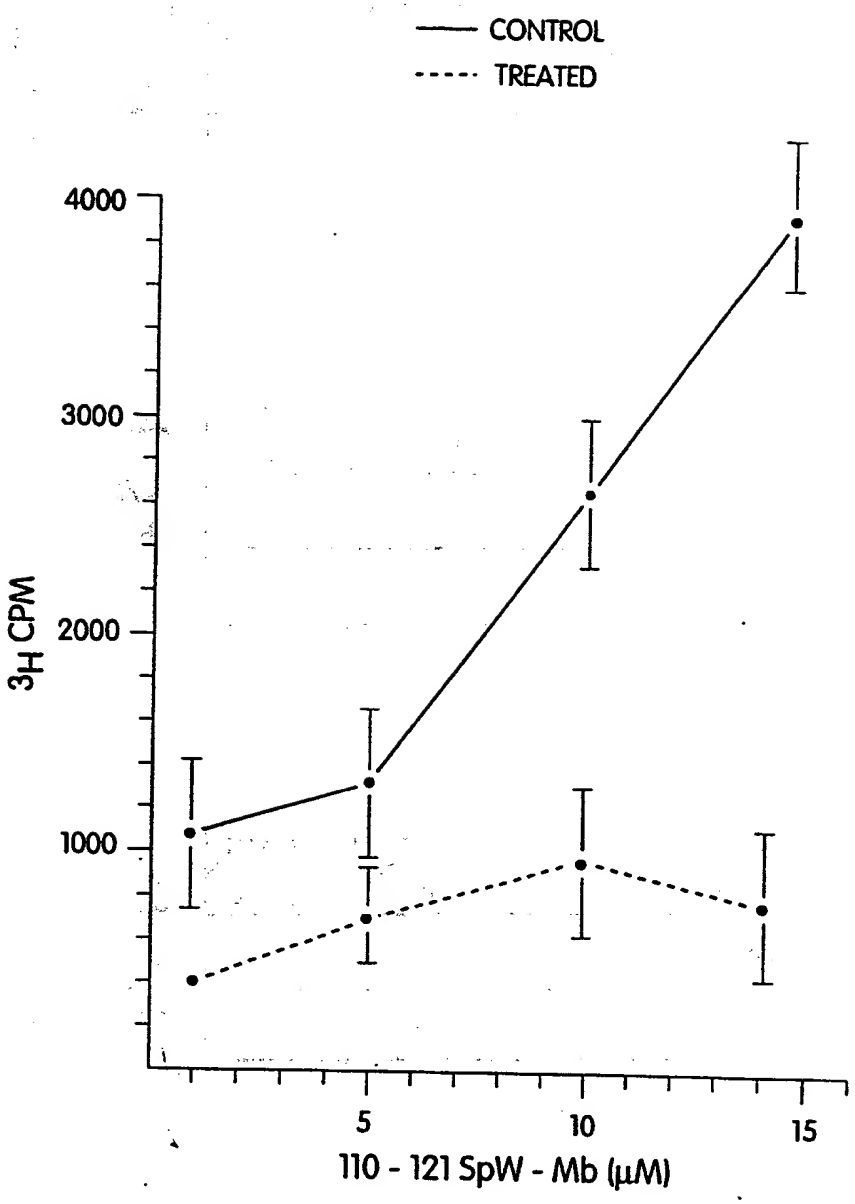


Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d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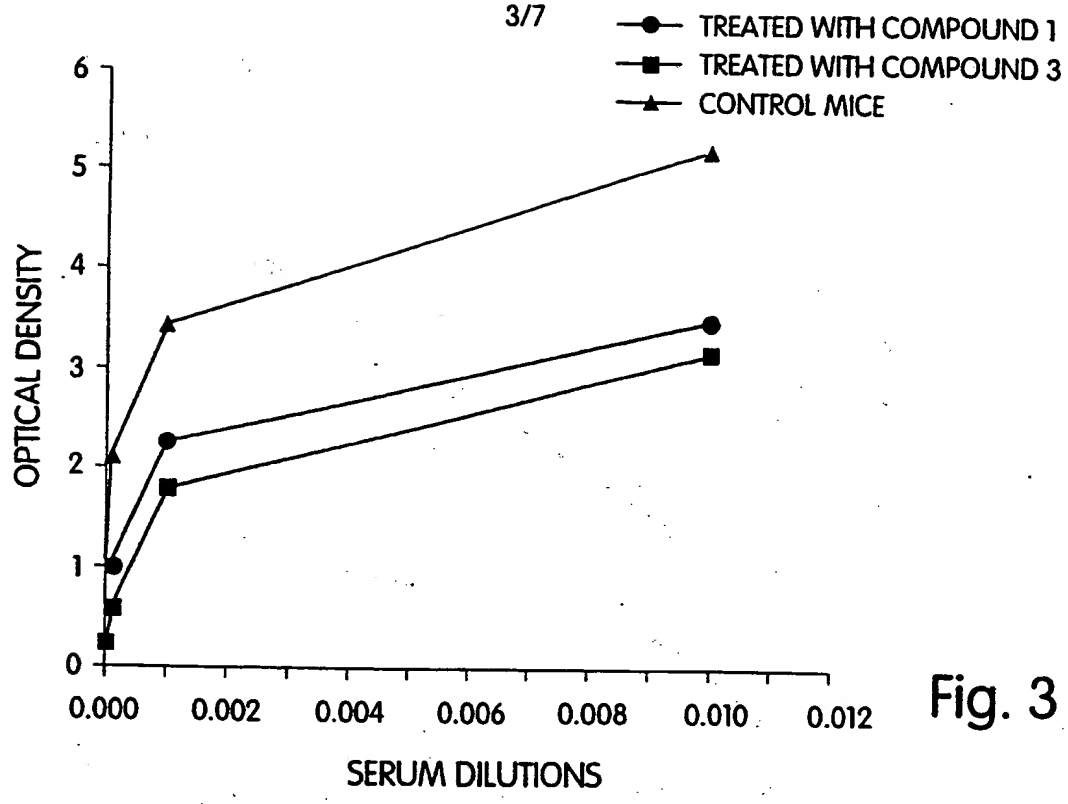
10
15
110 - 121 SpW - Mb (μM)

Fig. 2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TREATED WITH COMPOUND 3
CONTROL MICE
OPTICAL DENSITY
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2
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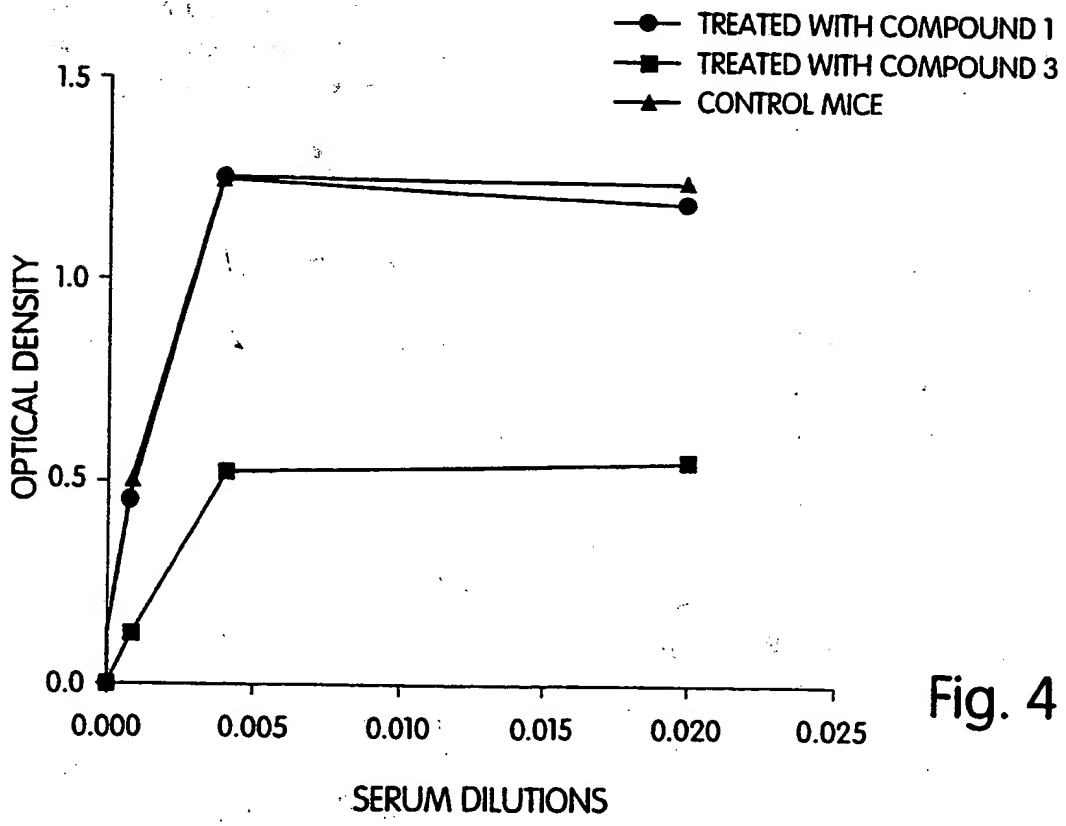
0.010
0.012
SERUM DILUTIONS

Fig. 3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TREATED WITH COMPOUND 3
CONTROL MICE
OPTICAL DENSITY
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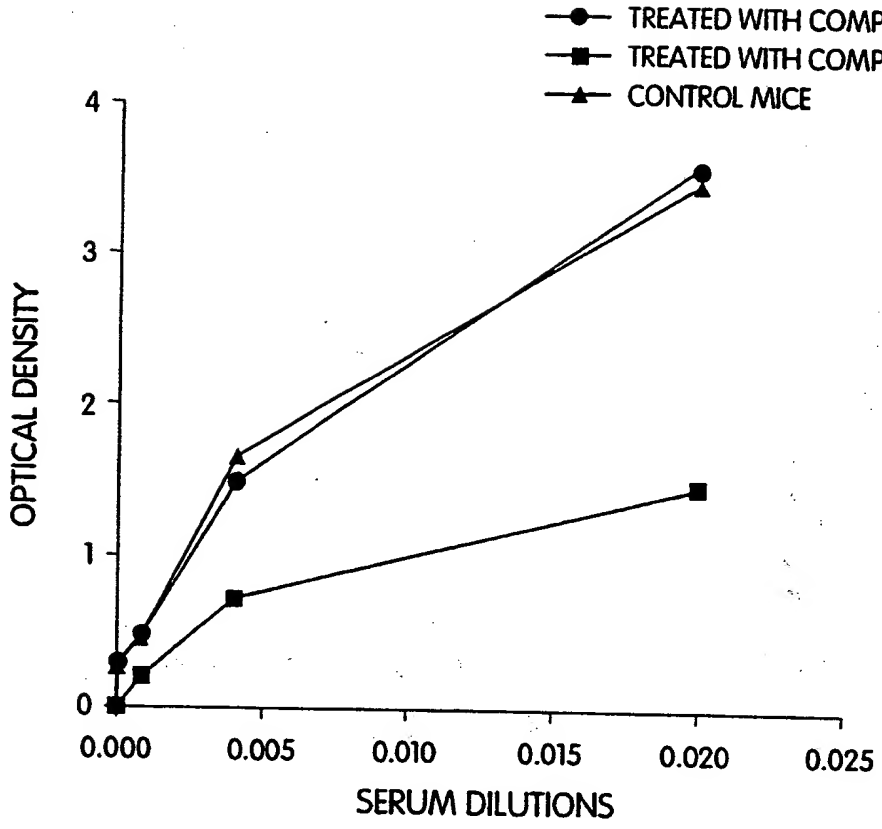
0.020
0.025
SERUM DILUTIONS

Fig. 4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TREATED WITH COMPOUND 3
CONTROL MICE
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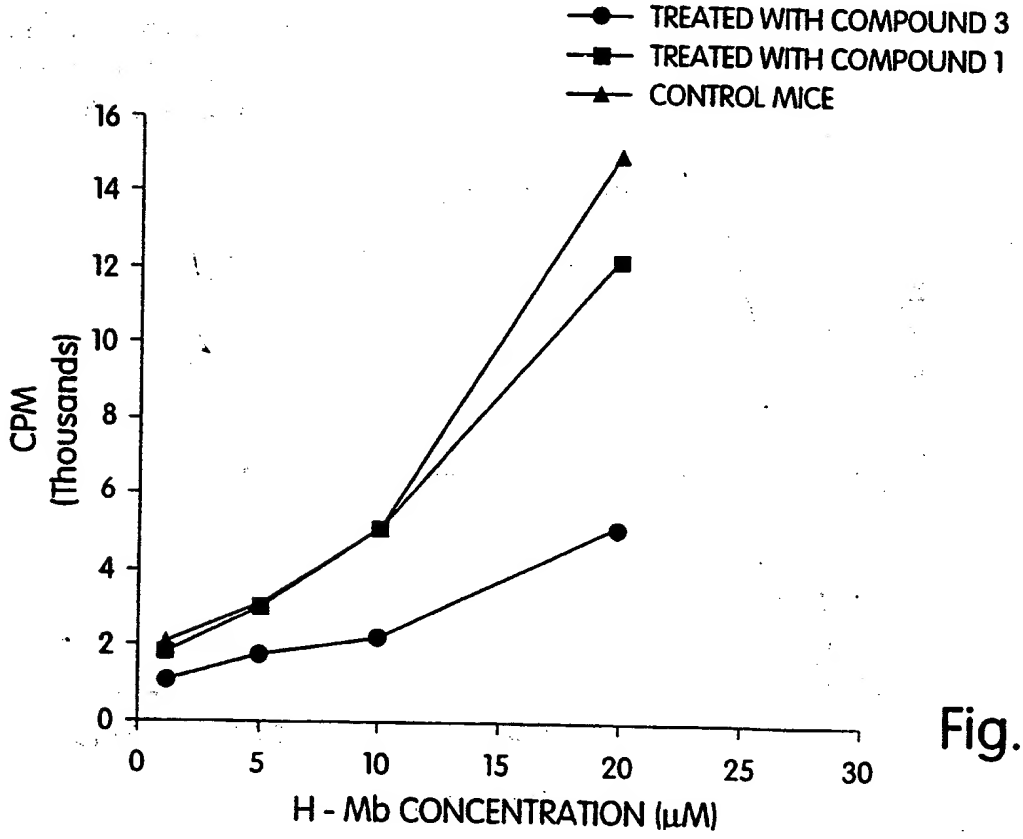
0.025
SERUM DILUTIONS

Fig. 5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CONTROL MICE
CPM
(Thousands)
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2
4
6
8
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12
14
16
0
5
10
15
20
25
30
H - Mb CONCENTRATION (µM)

Fig. 6

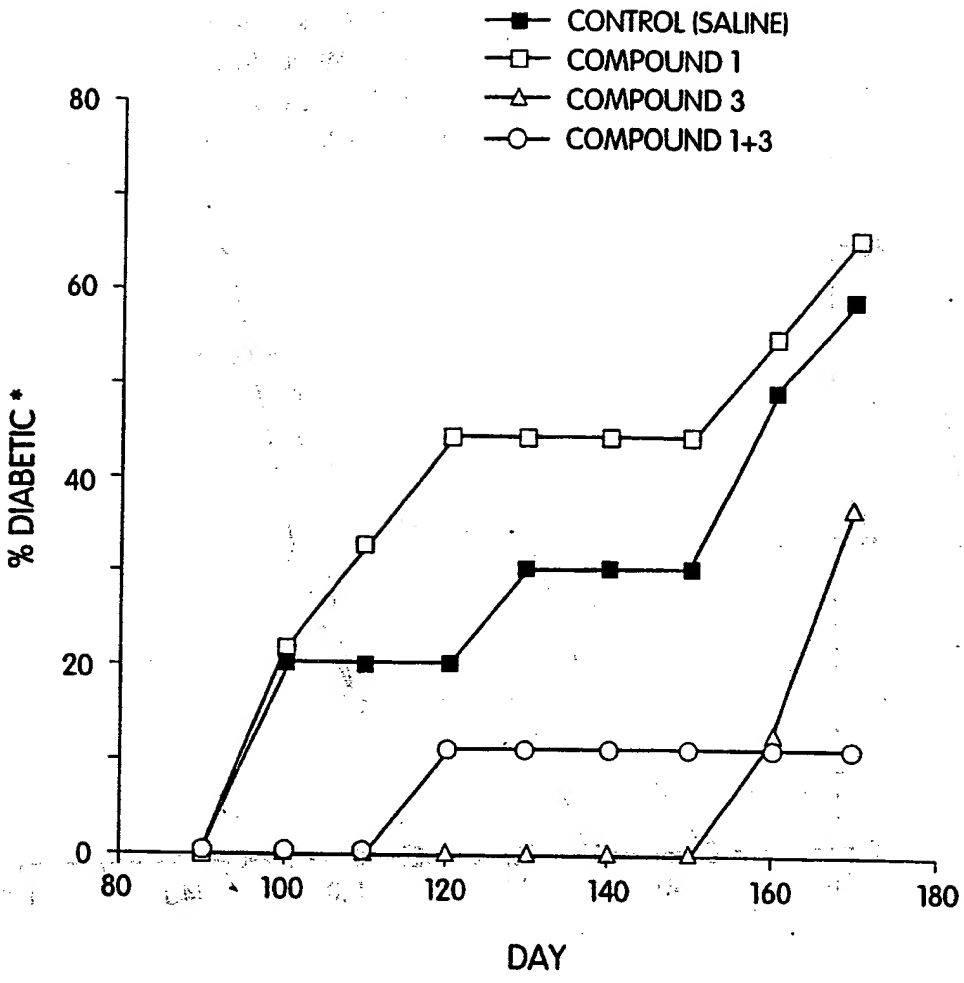
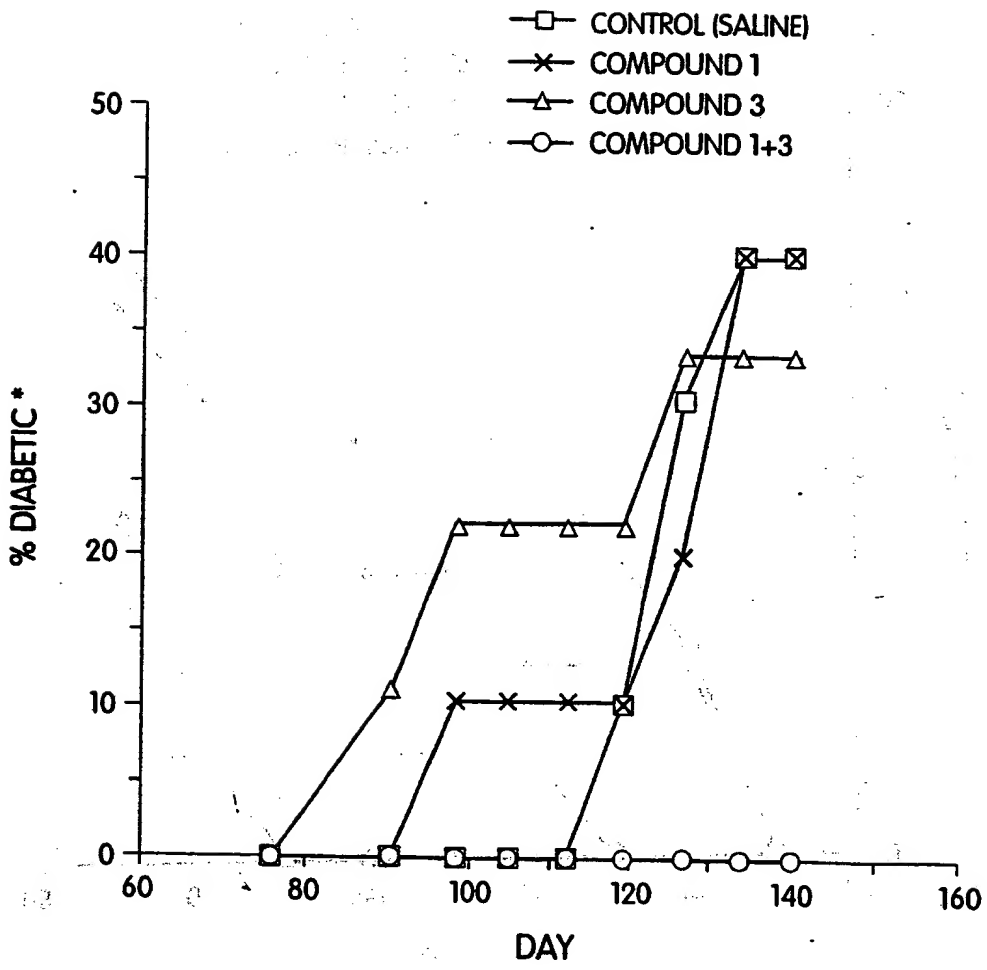
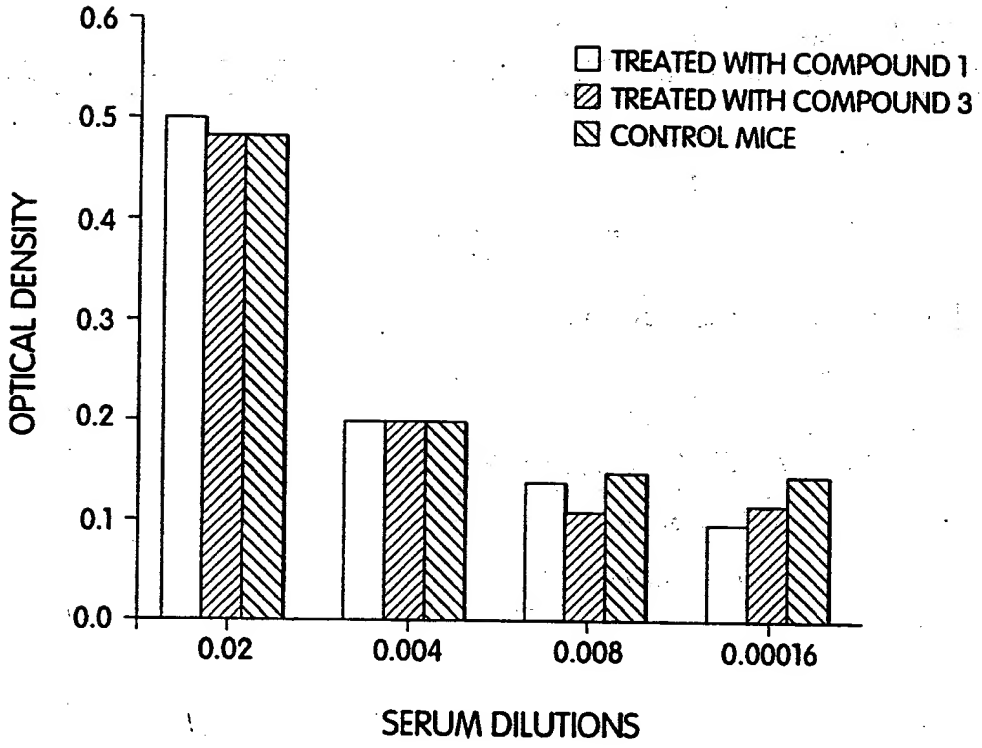

CONTROL (SALINE)
COMPOUND 1
COMPOUND 3
COMPOUND 1+3
% DIABETIC *
80
60
40
20
0
80
100
120
140
160
180
DAY
* # MICE >400 mg/dl GLUCOSE / TOTAL # IN GROUP x100

Fig. 7



* # MICE >400 mg/dl GLUCOSE / TOTAL # IN GROUP x100

Fig. 8


TREATED WITH COMPOUND 1
TREATED WITH COMPOUND 3
CONTROL MICE
OPTICAL DENSITY
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0.0
0.02
0.004
0.008
0.00016
SERUM DILUTIONS

Fig. 9

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00691

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/415

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	PROC. WEST. PHARMACOL. SOC. vol. 30, 1987, pages 383 - 387 M.M. KHAN ET AL 'Receptor and tissue specific derivatives of histamine: novel immune modulators.' see the whole document ---	1-27
Y	J. MED. CHEM. vol. 30, no. 11, 1987, pages 2115 - 2120 M.M. KHAN ET AL 'Congener derivatives and conjugates of histamine: synthesis and tissue and receptor selectivity of the derivatives.' see the whole document --- -/--	1-27

° Special categories of cited documents :[10]

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 MAY 1993	26. 05. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	KLAVER T.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	J. IMMUNOL. vol. 137, no. 1, 1986, pages 308 - 314 M.M. KHAN ET AL 'The effects of derivatives of histamine on natural suppressor cells.' see the whole document	1-27
Y	US,A,4 996 221 (MELMON ET AL) 26 February 1991 cited in the application	1-27

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9300691
SA 69798

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 07/05/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4996221	26-02-91	None	